31. The method of claim 9, wherein the protein-affinity chromatography is an automated process.

32. The method of claim 31, wherein the automated process includes procedures for preparing the columns and performing the affinity chromatography.

33. The method of claim 32, wherein the automated process includes procedures for packing the columns, coupling the protein ligand to the matrix, loading an extract onto the columns, washing the columns and eluting bound components from the columns.

34. The method of claim 9, further comprising correlative database searching with said peptide or peptide fragment masses, whereby the interacting protein is identified.

REMARKS

Applicants respectfully request entry of this amendment prior to examination of this case on the merits. Claims 1, 5, 8, 9, and 12 have been amended and new claims 16-34 have been added. This amendment enters no new matter. Support for the amended and added claims can be found throughout the specification as originally filed.

In particular, support for the amendment to claims 1 and 9 can be found, for example, at page 12, lines 12-13 and page 7, line 26.

Support for the amendment to claims 5 and 12 can be found, for example, at page 17, lines 4-10.

Support for new claims 16 and 30 can be found, for example, at page 11, lines 22-23.

Support for new claim 17 can be found, for example, at page 13, lines 3-5.

Support for new claims 18 and 29 can be found, for example, at page 12, lines 23-29.

Support for new claims 19-21 and 31-33 can be found, for example, at page 3, lines 31-32, page 13, lines 3-5, page 15, lines 23-26, page 16, lines 11-13 and 31-33, and page 18, lines 19-20.

Support for new claim 22 can be found, for example, at page 7, line 30.

Support for new claims 23-24 can be found, for example, at page 10, line 30 to page 11, line 2.

Support for new claim 25 can be found, for example, at page 11, line 32 to page 12, line 2.

Support for new claim 26 can be found, for example, at page 22, lines 23-24.

Support for new claim 27 can be found, for example, at page 13, lines 15-23.

Support for new claim 28 can be found, for example, at page 13, lines 25-30.

The cancellation and/or amendments to the claims are being made solely to expedite prosecution of the present application. Applicant reserves the option to further prosecute the same or similar claims in the instant or in a subsequent patent application.

CONCLUSION

If there are any fees due in connection with the filing of this response, please charge the fees to our **Deposit Account No. 06-1448**. If a telephone conversation with Applicant's Agent would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 832-1770.

Respectfully submitted,
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Marked-up version of claims showing changes made:

Please amend claims 1, 5, 8, 9 and 12 as set forth below.

1. A method for the identification of an interacting protein, said method comprising:

   a) subjecting an extract to protein-affinity chromatography on [multiple] two or more columns, said columns having a protein ligand [coupled to the column matrix] in varying concentrations immobilized to a matrix, and eluting bound components of said extract from said columns;

   b) separating said components to isolate an interacting protein;

   c) analyzing the interacting protein by mass spectrometry to identify the interacting protein.

5. The method of claim 4, wherein said polyacrylamide gel [does not] contains SDS.

8. The method of claim 1, wherein the bound components of the extract are eluted with a protein denaturant.

9. A method for the identification of an interacting protein, said method comprising:

   a) subjecting a cellular extract or extracellular fluid to protein-affinity chromatography on [multiple] two or more columns, said columns having a protein ligand coupled to the column matrix in varying concentrations, and eluting bound components of said extract from said columns;

   b) gel-separating said components to isolate an interacting protein; wherein the interacting protein is observed to vary in amount in direct relation to the concentration of coupled protein ligand;

   c) digestion of said interacting protein to give corresponding peptides

   d) analyzing said peptides by MALDI-TOF mass spectrometry or post source decay to determine the peptide masses[, and

   e) correlative database searching with said peptide or peptide fragment masses, whereby the interacting protein is identified].

12. The method of claim 11, wherein said polyacrylamide gel [does not] contains SDS.

Please add new claims 16-34 as set forth below.

16. The method of claim 1, wherein the protein ligand is immobilized to the matrix after the matrix has been packed into the column.

17. The method of claim 2, wherein multiple micro-columns are arranged into an array format.

18. The method of claim 1, wherein the columns are not blocked after immobilizing the ligand to the matrix.

19. The method of claim 1, wherein the protein-affinity chromatography is an automated process.

20. The method of claim 19, wherein the automated process includes procedures for preparing the columns and performing the affinity chromatography.

21. The method of claim 20, wherein the automated process includes procedures for packing the columns, coupling the protein ligand to the matrix, loading an extract onto the columns, washing the columns and eluting bound components from the columns.

22. The method of claim 1, wherein the protein ligand is at least 90% pure.

23. The method of claim 1, wherein the protein ligand is a fusion protein.

24. The method of claim 23, wherein the fusion protein comprises an affinity tag which may be used to couple the protein ligand onto the matrix.

25. The method of claim 1, wherein the concentration of the protein ligand bound to the matrix in at least one of the columns is at least 10-fold higher than the K_d of the interaction between the protein ligand and the interacting protein.

26. The method of claim 1, wherein the concentration of the protein ligand bound to the matrix is from 0 to about 2 milligrams of ligand per milliliter of matrix for all of the columns.

27. The method of claim 1, wherein the extract is derived from a tissue, cultured cell line, purified cellular organelle, or bodily fluid.

28. The method of claim 1, wherein the extract is a whole cell extract or a fractionated extract.

29. The method of claim 9, wherein the columns are not blocked after coupling the ligand to the matrix.

30. The method of claim 9, wherein the protein ligand is coupled to the matrix after the matrix has been packed into the column.

31. The method of claim 9, wherein the protein-affinity chromatography is an automated process.

32. The method of claim 31, wherein the automated process includes procedures for preparing the columns and performing the affinity chromatography.

33. The method of claim 32, wherein the automated process includes procedures for packing the columns, coupling the protein ligand to the matrix, loading an extract onto the columns, washing the columns and eluting bound components from the columns.

34. The method of claim 9, further comprising correlative database searching with said peptide or peptide fragment masses, whereby the interacting protein is identified.